claims can be found on p.10 line 27 to p.11, line 1; p. 22, line 24 to p. 23, line 3; and claims 47-51 of the originally-filed application.

REMARKS

Applicants thank Examiner Spiegler and Supervisory Patent Examiner Horlick for the discussions of October 22, 2001 and December 12, 2001. The substance of these discussions is incorporated into this Amendment. Issues bearing on 35 USC § 112 and 35 USC § 101 regarding the new claims were discussed and resolved.

Rejections presented in the Office Action of June 22, 2001 are moot due to the cancellation of the claims.

Status of the claims

Claims 57-66 are present and active in the application.

The Applicants have amended the claims and respectfully request their entry into the application. Claims 1-56 have been deleted, without prejudice to their prosecution in subsequent continuation or divisional applications. Claims 57-66, directed to inhibiting cell differentiation, are now pending.

Pluripotent stems cells differentiate to many different cell types. For example, stem cells harbored in bone marrow give rise to hematopoietic cells, such as eosinophils, basophils, neutrophils, megakaryocytes (giving rise to platelets), and erythrocytes. A major problem impeding progress in stem cell therapies is the accumulation of pure cultures of stem cells. However, inhibiting differentiation of a stem cell confers the advantage of accumulating many stem cells in a pure culture *in lieu* of cultures having various differentiated populations that arise spontaneously *in vitro*. Such an accumulation of undifferentiated stem cells can be especially beneficial in cell replacement therapies, thus providing a generous source of cells ready for transplanting. Such an approach can be further exploited, for example, by using regenerative stem cells found in adult tissues that have the potential to differentiate into hematopoietic stem, muscle, liver and pancreas cells (Brill et al., 1993; Jackson et al., 1999; Ramiya et al., 2000;

Seale and Rudnicki, 2000); thus inhibiting stem cells from differentiating to better control their response to differentiating factors, enabling enriched cultures of newly-differentiated cell types. Inhibiting cell differentiation can also be used to *direct* cell differentiation. For example, stem cells can be activated to pursue various avenues of differentiation by either extracellular factors alone or in combination with timing (the age of the cell). Finally, inhibiting stem cell differentiation can be used to unravel the complexities of hematopoiesis: "The behavior of pluripotent stem cells [discussed in the context of hematopoietic stem cells] remains especially elusive; these crucial cells are few and far between—less than 1 in 1000 of the cells in the bone marrow..." (Alberts et al., 1994). Finally, a population of stem cells may be synchronized not only at the level of the cell cycle, but now also at the level of differentiation.

The invention solves these problems, by inhibiting stem cell differentiation to prepare large pure stem cell cultures, using polypeptides comprising at least 85% sequence identity to the sequences of HEMA1 or HEMA2 (SEQ ID NOs:2, 4, 5 or 6).

The present invention meets all of the utility requirements of 35 USC § 101; the invention is (1) credible, (2) specific and (3) substantial. The invention is credible, as shown by the experimental evidence on p. 3, line 24 through p. 8: HEMA1 and HEMA2 polypeptides inhibit differentiation when contacted with a stem cell. In this case, cells producing HEMA1 and HEMA2 polypeptides are administered *via* a co-culture system of the experiment. Background to these types of experiments is presented below in *Background of cell lines* and (Ohneda et al., 1998). The invention is specific. The invention provides methods directed to inhibiting stem cell differentiation using HEMA1 and HEMA2 polypeptides (p. 10 line 27 to p.11, line 1; and p. 22, line 24 to p. 23, line 3). Tthe utility is not a general application of polypeptides. Finally, the invention is substantial, immediately providing methods to inhibit stem cell differentiation, thus enabling the preparation of pure cultures. The utility of the invention is tremendous, especially given the current fast-paced research centered on stem cells for therapeutic use.

Background of cell lines

The discovery of the roles of HEMA1 and HEMA2 in cellular differentiation was elucidated in experiments designed to define hematopoietic regulatory factors. These regulatory factors were determined from a differential-expression based assay, GeneCalling, using three known endothelial cell lines, DAS 104-4, DAS 104-8 and YS CL72 (Ohneda et al., 1998). The two DAS cell lines were sub-cloned from "DAS 104", which expresses a variety of endothelial markers and forms capillary-like tubes when suspended in basement membrane from EHS tumors, (a classic *in vitro* test for angiogenic activity) (Ohneda et al., 1998). The intention of developing these cell lines was to determine their capabilities regarding stem cell differentiation. These cells lines were created by first isolating CD34-expressing cells (CD34 is an antigen expressed mostly by endothelial and hematopoietic stem cells in mouse embryos) from the *a*orta-gonad-*m*esonephros (AGM) region of mouse embryos. Endothelial cells were removed by exploiting their innate adherent properties. After expansion, these primary cells were again analyzed for CD34 expression by fluorescence-activated cell sorting (FACS), pooled, and then immortalized by polyoma virus middle-T transformation. Subcloned lines were then sorted again by FACS, selecting CD34 expression-positive cells. Two subclones were selected and analyzed: DAS 104-4 and DAS 104-8. Their characteristics are summarized in Table A.

The YS CL72 cell line is a day 10.5-yolk sac-derived endothelial cell line (Fennie et al., 1995). This cell line was isolated and transformed by similar methods that were used to isolate the DAS cell lines; like the DAS cell lines, YS CL72 form capillary structures when suspended in a basement membrane gel. Subculture with yolk sac CD34+ hematopoietic cells results in up to a 60-fold increase in total hematopoietic cell number; these expanded cells were mostly of monocyte/macrophage lineage. This cell line also has the ability to expand committed progenitor erythroid and myeloid cells. Exogenous erythropoietin induces the output of erythroid cells from the mature hematopoietic cells from co-culture with YS CL72.

Table A Characteristics of DAS 104-4, DAS 104-8 and YS CL72 (Ohneda et al., 1998)

Effect on hematopoietic stem cells in co-culture	**DAS 104-4**	**DAS 104-8**	**YS CL72**
Summary	Expansion and	Expansion and	Expansion and

	differentiation of hematopoietic stem cells from fetal liver; mostly myeloid-like cells	differentiation of hematopoietic stem cells from fetal liver; mostly myeloid-like cells	differentiation of yolk sac CD34+ hematopoietic cells to myeloid and erythroid cells
Stem cell differentiation induction	**Less** differentiated stem cells when compared to DAS 104-8 co-cultured cells	**More** differentiated stem cells than when co-cultured with DAS 104-4 cells	Not directly compared to DAS cell lines in (Ohneda et al., 1998)
cell-cell contact necessary for stem cell effects?	Yes	Yes	-
co-culture with erythropoietin (EPO)	Induces some stem cells down the erythroid pathway of cellular differentiation	Induces many stem cells down erythroid path	Increases the number of erythroid cell population from mature hematopoietic cells
co-culture with Interleukin-7 (IL-7)	Stem cells were not induced to differentiate to other cell types	Cells differentiate, mostly B cells	n.d.
Mechanism of expansion and maintenance of differentiation	Maintains undifferentiated hematopoietic stem cells and expands them	Does not maintain hematopoietic stems cells as undifferentiated and is incapable of expanding them	Maintains hematopoietic stem cells and expands them
Replace bone marrow	Competent	Incompetent	-

In the experiments that gave rise to the compositions and methods of the invention, the applicants took advantage of the DAS 104-4 and DAS 104-8 cell lines, with YS CL72, isolating those molecules that are up- or down-regulated.

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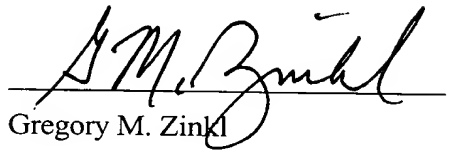
Applicants submit that the present application is now in condition for allowance. Early notification to such effect is solicited.

Respectfully submitted,



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